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Perspectives on engineering organs *in vitro*: overcoming oxygen supply limitations

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The shortage of compatible organs in comparison to the increase of the aging population and the number of patients with traumatic injuries, stresses the necessity of fabricating or regenerating organs or tissues *in vitro* aimed at replacing or repairing therapies. Tissue Engineering (TE) is a branch of Regenerative Medicine based on the joint application of biomaterials, mammalian cells and bioactive molecules (also known as growth factors) to produce functional tissues or organs. The concept of TE was first introduced in the earliest 1990's [1]. In order to favor and guide the cell differentiation into three-dimensional (3-D) tissues, the cells need to be cultured on porous scaffolds that mimic the functions of the extracellular matrix (ECM), the natural scaffolding of the living tissues. These artificial scaffolds should, therefore, accomplish with certain requisites, for instance, i) they must be fabricated using biocompatible materials that provides with a chemically favorable environment for the cell adhesion and proliferation and avoids or limits the inflammatory response typically produced by external agents on any animal body, ii) the morphology and topography of the scaffold surface should also be attractive to the cells and iii) the material must have sufficient mechanical properties. A large number of scientific contributions have been dealing with the development of scaffolds with adequate properties to fabricate tissue constructs under *in vitro* conditions.

Despite the efforts invested in the development of suitable scaffolds, the major difficulty to grow mammalian cells with sufficient density to produce functional and fair-sized tissues *in vitro* has been attributed to the important limitations of nutrients supply (e.g. glucose and oxygen) and metabolites removal between the cell tissues and the culture medium. This problem importantly arises when cell seeded-scaffolds are cultured statically in petri dishes due to nutrients depletion with time and convection and diffusion mass transport

limitations of the nutrients in the surroundings of the scaffold. This observation transformed the initial materials engineering approach into a mass transport engineering obstacle.

Oxygen is frequently the most limiting nutrient in cell cultures *in vitro*. Cells consume oxygen between 1 to 6-fold the amount of glucose (depending on the tissue type), albeit the concentration of glucose in the culture medium is approximately 100 times higher than the oxygen one [2]. This shortage of oxygen availability restricts the maximum size of 3-D tissues that can be produced *in vitro*. For example, some predictions established a maximum tissue thickness of 1 mm when using blood as non-circulating fluid. When tissue constructs present highersize than 1 mm, the restrictions of oxygen diffusion into the 3-D matrix causes hypoxia in the tissue constructs.

Different bioreactor designs introducing stirring or rotation have been developed in order to reduce the convectional resistances of the nutrients in the proximities of the cell constructs [3]. However, with these configurations the diffusional resistance still persists due to the difficulty of the tissue constructs on developing neovascularization. The solution found was to incorporate perfusion of the culture medium through the cell constructs. Particularly, hollow fiber perfusion bioreactors (HFPB) simulated an artificial vascular network and minimized the shear stress of the flowing culture medium on the seeded cells resulting in high 3-D cell density cultures [4]. Still certain size limitations remain using HFPB and complex designs improving the classical HFPB configuration have been proposed to emulate the intricate and branched structure of the natural blood capillaries network.

Apart from the kinetic parameters (convectional and diffusional) affecting the mass transport of oxygen from the culture medium to the cell constructs, the driving force of the process, that is, the oxygen concentration gradient between the culture medium and the cells that consume it, needs alsoto be considered. The oxygen concentration in the culture medium reaches a maximum value determined by its solubility limit (approx. 0.2 mmol O_2/L) in this aqueous phase. However, oxygen concentration in the blood of a healthy human male adult can reach 9.5 mmol O_2/L due to the presence of the oxygen-binding molecule hemoglobin in the blood [2]. Some authors propose the incorporation of oxygen-carrier molecules supplemented in the culture medium to increase the oxygen concentration in the feeding phase [5]. Examples of these type of molecules are hemoglobin-based and perfluorocarbon compounds. On the one hand, the hemoglobin-based molecules bind chemically the oxygen similarly as the hemoglobin protein of the red blood cells but lack the cellular component and its associated drawbacks (i.e. immunological responses, blood type incompatibilities, potential infection transmissions, low storage stability). On the other hand, the perfluorocarbons have a high O_2/CO_2 solubility (approximately 20 times higher than water) and is extremely inert and stable, however, these molecules are not soluble in water and their incorporation in the culture medium should be done in emulsion.

Other alternative presented in the literature to increase the oxygen availability in the bioreactors is the preparation of oxygen-releasing biomaterials [6]. The most common oxygen-releasing molecules are e.g. sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide and fluorinated compounds. Different methods could be used to incorporate these oxygen-releasing molecules into biomaterials, such as, adsorption or encapsulation.

In short, the research in TE aiming at fabricating large functional tissues and organs under *in vitro* conditions has benefitted in the last years of many engineering tactics and ideas to come closer to the success. Promising results have been observed in all the strategies aforementioned to overcome the limitation in oxygen supply in bioreactors to produce fair-sized 3-D cellular constructs. The door has been already opened but there is still a long way to produce a breakthrough in the TE field to achieve its ultimate goal.

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